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LEVELS OF HEAVY METALS AND PHYSICOCHEMICAL PROPERTIES OF HONEY FROM FOUR SELECTED AREAS OF ETHIOPIA

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ABSTRACT. In this work, the qualities of honey samples were evaluated by assessment of some physicochemical parameters and levels of heavy metals (Cd, Pb, Cr, Ni, Zn and Cu). The honey samples were collected from the major producing regions of Ethiopia; Gojjam, Gondar, Jimma and Tigray. The pH and electrical conductivity (EC) of the samples were determined using digital pH-meter and conductivity meter, respectively. The metal contents were determined by flame atomic absorption spectrometry (FAAS). The validity of the method employed was evaluated by spiking experiments with recoveries ranging from 90.0 to 95.8%. The FAAS results show higher concentration of Cu and Zn compared to other metals. The amount of Cu and Zn were in the range 6.2–18 µg/g and 4.2–10 µg/g. The heavy metals (Cd, Pb, Cr and Ni) were below detection limit in all the honey samples. Moreover, the result obtained regarding the physicochemical properties showed that the pH of all the honey samples were acidic (pH of 3.84–4.00), the EC 0.09–0.34 $mScm^{-1}$ and the ash content 0.13–0.33%. The results obtained were in agreement with European standard and Codex Alimentarius, indicating the honey samples studied are of good quality and suitable for human consumption.

KEY WORDS: Honey, Heavy metals, Physicochemical properties, FAAS, Ethiopia

INTRODUCTION

Honey is a natural sweet and viscous substance produced by honey bees (*Apis mellifera)* from the nectar of blossoms or secretions on living plants [1-3]. The bees collect this sugary substance deposit, reduce the water content, store and leave in honey combs or honey pots to ripen and mature for their own consumption [4]. Honey is a recognized natural food worldwide which has high nutritional value and many beneficial health promoting effects [5]. The composition and properties of honey are highly variable depending on its sort, origin, environment, harvest time, climatic conditions, and treatment of beekeepers [6-10]. Honey can be classified by its floral nectar source. The flowers from which bees gather nectar largely determine the color, flavor and aroma. Honey can be mono-p, poly-floral or blended [11].

Honey is mainly composed of carbohydrates (80–85%), water (15–17%), protein (0.1–0.4%) and traces of organic acids, enzymes, amino acids and phenolic compounds which contribute greatly to its sensory and functional characteristics [12-14]. Honey also contains minerals and heavy metals, which play important roles in determining the quality of honey. The mineral content varies, ranging from 0.04% in pale honeys to 0.2% in darker honeys [15]. The major minerals are derived mainly from the soil and nectar producing plants, but they may also originate from environmental contamination. Based on the reports of Solayman and his co-workers [15], trace minerals coming from organic or plant sources are beneficial for maintaining human health, while heavy metals which come from various anthropogenic factors, primarily from the environmental pollution can have detrimental effects to human health.

According to the reports of [16-18], Ethiopia is the major honey producer in Africa. It owns a big potential for honey production due to its varied ecological and climatic conditions. The honey

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production accounts for approximately 21.7% of Africa and 2.5% of world production, respectively [17]. Honey is produced in almost all parts of Ethiopia. However, the most important honey producing regions are Oromia (38%), followed by Amhara (26%), Southern Regional States, (18%), Tigray and Benshangul-Gumuz (8% and 7%, respectively) of the total honey production [19].

Trace metals such as Zn, Cu, Ni, etc. are common in honey and beneficial to human health in trace amounts. However, some trace heavy metals (Pb, Cd, and Cr) are non-essential and toxic in trace or large amount and have the potential to affect the quality of honey and their presence can harm human health through the food chain [20].

Therefore, it is important to evaluate the quality and composition of honey to determine its suitability for human consumption and to meet the demands of the market. Recently, some research works were reported on honey samples from different areas in Ethiopia. For example, the effect of heavy metal and storage time on hydroxymethylfurfural formation in honey by Birhanu and Tolcha [1]; the determination of levels of trace heavy metals and physicochemical properties of honey by Tibebe *et al.* [20]; antimicrobial activities and physicochemical characteristics of honey by Nemo, and Bacha [21]; the investigation of the effects of season and location on concentration of metals by Engidaw *et al.* [22] have been reported.

However, there is no enough data available, which gives us comprehensive information about the levels of trace and toxic metals and quality physicochemical parameters in honey collected from the potential honey producing areas in Ethiopia. The present work is relevant to complement the works done so far and fill the gap.

In the present study, selected quality physicochemical parameters namely: ash content, pH, electrical conductivity and trace heavy metals (Pb, Cd, Zn, Cr, Cu and Ni) of honey samples collected from the major honey producing areas of Ethiopia, i.e. Gojjam, Gondar, Jimma and Tigray were determined and compared with reports in the literatures and international standards.

EXPERIMENTAL

Chemicals and reagents

All the reagents used were of analytical grade. A mixture of concentrated HNO_3 (69–72%, Spectrosol, BDH, England) and concentrated $HClO_4$ (70%, BDH Laboratory Supplies AnalaR®, Poole, England) were used for digestion of honey samples. Stock standard solutions containing 1000 mg/L in 2% HNO_3 (BDH Chemicals Ltd Spectrosol®, Poole, England) were used for the preparation of working calibration standard solutions and for spiking. Deionized water (chemically pure < 1.5 μscm^{-1}) was used throughout the experiment for sample preparation, dilution and rinsing apparatus.

Apparatus and equipment

Atomic Absorption Spectrophotometer (ZEE nit 700P, Germany); 250 mL round bottom flasks fitted with reflux condenser and Kjeldahl digestion block apparatus (Gallenkamp, England) were used to digest spiked and non-spiked honey samples and blank solutions; refrigerator to keep the collected samples and digested samples until analysis, digital balance (Mettler Toledo, Model AG204, Switzerland) to weigh honey samples, digital pH meter(pH/Ion Level 2, Germany) and conductivity meter (HANNA Instruments, Portugal) to measure pH and electrical conductivity, respectively, electrical furnace (Audiotronics, Wagtech International Ltd., UK) for ashing the honey samples, water bath to liquefy honey samples.

Sample collection and preparation

Four different honey samples were purposively collected from the major honey producing regions of Ethiopia Tigray (Adigrat), Gojjam (Debre Markos), Jimma and Gondar. Honey samples were prepared in accordance with AOAC Official Method [23]. All the honey samples were randomly collected from beekeepers. It is floral honey. It could be possibly poly or mono floral honey type. All the honey samples weighing about 500 g were randomly collected from beekeepers during the main harvesting season (October to November, 2018). From one particular sampling area, three beekeepers were considered. The honey samples from the beekeepers of similar area were mixed together to get a composite sample (about 500 g) representing each sampling area. The honey samples were then stored in clean polyethylene jars and kept in a refrigerator until analysis. Then, analysis of each sample was carried out in triplicate for determining the physicochemical properties (ash content, electrical conductivity, pH) and the levels of trace heavy metals (Cd, Pb, Cr, Ni, Zn and Cu).

Honey samples were prepared in accordance with AOAC official method 920 180 [23]. Accordingly, the honey samples were heated to 65 °C in a water bath until liquefied to permit easier handling to decrease viscosity for more uniform distribution. The samples were then cooled and weighed for subsequent analysis.

Determination of ash content

Determination of ash content was done according to the AOAC official method 920 181 [24]. Honey samples of 5 g was placed in previously weighed porcelain crucible and dried on a hot plate until the sample was turned into black and dry [24]. This was done to remove moisture content that would cause foaming of the honey during the early stages of ashing. After removing the crucibles from the hot plate, they were cooled in desiccators for about 4 hours and weighed. The materials were then ashed in an electrical furnace at 600 °C for 6 hours, followed by cooling in desiccators and then weighed [25].

Determination of pH and EC

The pH of honey was determined according to the method adapted from the ref. [18]. 10 g of each honey sample was diluted with 75 mL deionized water. The pH was measured using a digital pH meter which was calibrated at room temperature using buffer solutions at pH 4 and 7. The electrical conductivity of samples was measured by using a conductivity meter directly.

Digestion of honey samples

Kjeldahl digestion block apparatus was used to digest honey samples. In the digestion of honey samples, good optimum condition is obtained when clear and colorless solution is formed [18]. Accordingly, 0.5 g of honey sample was accurately weighed on a digital analytical balance and transferred quantitatively into a 250 mL round bottom digestion flask. 2 mL of conc. HNO_3 (69-72%) and 2 mL of conc. $HClO_4$ (70%) was added to the sample. The sample was swirled gently to homogenize the mixture then it was fitted to a reflux condenser and digested continuously for 3 hours on a Kjeldhal digestion block by setting the temperature at 240 °C until clear and colorless solution was obtained [18].

Each honey sample was digested in triplicate and a total of 12 digests were made for the four types of honey samples collected from four different areas of Ethiopia. The samples were cooled to room temperature for 15 min without removing the condenser from the flask and for 15 min more after removing the condenser. To the cool solution, deionized water was added to dissolve the precipitate formed on cooling and to minimize dissolution of the filter paper by the digest

residue while filtering with filter paper. The round bottom flasks were rinsed subsequently with deionized water into 50 mL volumetric flasks and finally the volumetric flasks were made up to the mark with deionized water. The digest was clear and colorless solution. For the analysis of the honey samples, 6 reagent blank samples were prepared. All the digested samples were stored in a refrigerator until analysis.

Determination of the metals

Intermediate standard solutions (10 mg/L) of metals of interest were prepared from the 1000 mg/L standard stock solutions. These intermediate standards were diluted with deionized water to obtain four working standards of each metal (Cd, Pb, Zn, Cr, Ni and Cu) and analyzed after the instrumental operating conditions were optimized for maximum signal intensity of the instrument. Triplicate determinations were carried out on each sample. Hallow cathode lamp for each metal, operated at the manufacturer's recommended conditions was used at its corresponding primary source line.

The correlation coefficients (R^2) of the calibration curves were determined by plotting the prepared working standard concentrations versus their corresponding absorbance. The correlation coefficients obtained were in the range of 0.995-0.999, indicating there was a good correlation between concentration and absorbance. The wavelength, detection limits of the instrument, working calibration standard concentrations, correlation coefficients and equations for the calibration curves for the determination of metals in honey samples using FAAS are shown in Table 1.

Table 1. Wavelengths, detection limit, working standard concentration, correlation coefficients and equation of the calibration curves for the determination of metals in honey using FAAS.

Metals	Wave length (nm)	Detection limit (mg/L)	Working standard concentrations (mg/L)	Correlation coefficient (R^2)	Equation of the calibration curves
Cd	228.8	0.012	0.25, 0.50, 0.75, 1.0	0.997	y = 0.049x - 0.001
Pb	283.3	0.03	0.4, 0.8, 1.2, 1.6	0.995	y = 0.003x + 0.0003
Zn	213.9	0.012	0.25, 0.50, 0.75, 1.0	0.995	y = 0.090x + 0.0001
Cr	357.9	0.05	0.5, 1.0, 1.5, 2.0	0.999	y = 0.011x - 0.0002
Ni	232.0	0.07	0.5, 1.5, 3.0, 6.0	0.999	y = 0.006x - 0.0001
Cu	324.8	0.035	0.25, 0.5, 1.0, 2.0	0.999	y = 0.024x + 0.0002

Recovery test

To check the validity of the digestion method employed, spiking experiments were carried out. Known amount of the metals were added from the intermediate standard 10 mg/L of solution into flasks containing 0.5 g of the sample. The spiked samples were digested simultaneously with the un-spiked samples. Each sample was then analyzed for their respective spiked metals by FAAS. The results are given in Table 2. The percentage recovery was calculated by:

$$\% \text{ Recovery} = \left(\frac{C2-C1}{C}\right) \text{x}100\%$$

where; C2-metal content of the spiked sample, C1-metal content of un-spiked, C-content of metal added.

As shown in Table 2, the results of percentage recoveries for metals in honey were between 90.0% and 95.8%. These are within the acceptable range for the metals, indicating the validity of the digestion method used for the honey samples.

Table 2. Recovery test results for honey samples.

Metals	Conc. in sample (mg/L)	Amount added (mg/L)	Conc. in spiked sample (mg/L)[a]	% Recovery [b]
Cd	BDL	0.30	0.28 ± 0.011	93.3 ± 2.2
Pb	BDL	0.50	0.47 ± 0.013	94.0 ± 3.5
Zn	0.097 ± 0.009	0.036	0.131 ± 0.01	95.8 ± 7.3
Cr	BDL	0.60	0.54 ± 0.012	90.0 ± 3.2
Ni	BDL	0.60	0.55 ± 0.01	91.6 ± 3.1
Cu	0.078 ± 0.015	0.028	0.103 ± 0.014	90.7 ± 4.4

[a]concentration means ± SD of triplicate readings. [b]% Recovery means ± SD of triplicate measurements.

Method detection limit (MDL) and limit of quantification (LOQ)

MDL is the minimum concentration of analyte that can be measured and reported with 99% confidence that the analyte concentration is greater than zero, but it may not necessarily be quantified as an exact value [26]. The MDL values were obtained by multiplying the standard deviation of blank solution by three while the LOQ values were found by multiplying the standard deviation of the blank solution by ten times. MDL, LOQ and instrumental detection limits are given in Table 3. The method detection limits for Zn and Cu are 0.164 and 0.106 µg/g, respectively, which indicated that the method used is applicable to determine the metal concentration in the honey samples at trace levels. As shown from the table, the MDL of Zn and Cu are higher than the instrumental detection limit. Therefore, the method used is applicable to determine the metal concentration in honey samples by using FAAS.

Table 3. Method detection limit, limit of quantification and instrumental detection limits.

Metals	Method detection limit (µg/g)	Limit of quantification (µg/g)	Instrumental detection limit (µg/g)
Zn	0.164	0.547	0.012
Cu	0.106	0.353	0.035

RESULTS AND DISCUSSION

Ash content

The ash content in honey is generally low (Table 4). It is influenced by the chemical composition of the nectar that varies according to the different botanical sources involved in honey formation. It can vary between 0.02 and 1.0% and the maximum limit allowed by European Legislation [27] and Codex Alimnetarius [28] for honey from floral sources is 0.6% and 0.8%, respectively.

Higher mineral contents about 1.0% are actually found only in honeydew honey and ash content is often used to identify the honey source [19, 20]. The ash content is expressed as percent ash (mass/mass) [21].

Table 4. Ash content, pH and EC of the four honey samples (mean ± SD).

Honey samples	Ash content (% w/w)	pH	EC ($mScm^{-1}$)
Gojjam	0.20 ± 0.00	3.91 ± 0.04	0.14 ± 0.01
Gondar	0.33 ± 0.01	3.84 ± 0.00	0.34 ± 0.01
Jimma	0.13 ± 0.01	3.85 ± 0.02	0.09 ± 0.00
Tigray	0.26 ± 0.01	4.00 ± 0.00	0.18 ± 0.00

The values of ash content for the studied honey samples from Gojjam, Gondar, Jimma and Tigray are 0.2%, 0.33%, 0.13% and 0.26%, respectively as shown in Table 4. Honey from Gondar has the highest ash content and electrical conductivity, indicating high mineral content in these samples. These vales indicate the accumulation of minerals with time in the honey samples. The ash contents of the honey samples in this study were all within the permissible limits established by European Legislation [27] and Codex Alimnetarius [28], indicating the honey samples studied are of floral origin.

pH and electrical conductivity

Honey is naturally acidic due to the presence of organic acids (gluconic acid, tartaric, citric, oxalic, acetic, etc.), nectar or bees secretions that contribute to honey flavour and inhibition against the growth of bacteria and other micro-organisms [29, 30].

In this study, as shown in Table 4, the pH values of all honey samples were found to be acidic. The mean pH values were; for Gojjam 3.91, for Gondar 3.84, for Jimma 3.85 and for Tigray 4.00. The pH was within the accepted range (3.5-5.5) according to the Codex Alimentarius Commission, 2001 [28]. The slight variation in the pH values of the studied honeys may be attributed to the nectar pH, differences in the soil composition or the differences in the plant species [31].

The electrical conductivity (EC) is another important factor for determining the physical characteristics of honey. EC depends on predominantly on the mineral content of honey. It is a physicochemical parameter that exhibits great variability based on the floral origin and is considered one of the best parameters to distinguish between honeys with different floral origins [32, 33].

The EC values of the honey samples recorded were: for Gojjam 0.14 $mScm^{-1}$, for Gondar 0.34 $mScm^{-1}$, for Jimma 0.09 $mScm^{-1}$ and for Tigray 0.18 $mScm^{-1}$. In the Codex Alimentarius, the maximum EC for pure floral honey is 0.8 $mScm^{-1}$ [28]. The electrical conductance of Gojjam, Gondar, Jimma and Tigray honey samples were found lower than that established by the Codex Alimentarius [28] (< 0.8 $mScm^{-1}$), indicating that the studied honeys are possibly floral honeys than honeydew origin. Similar results were obtained by Nigussie *et al.* for honey samples from Tigray [33].

In this study, a linear relationship between the ash content and the electrical conductivity was observed (Figure 1).

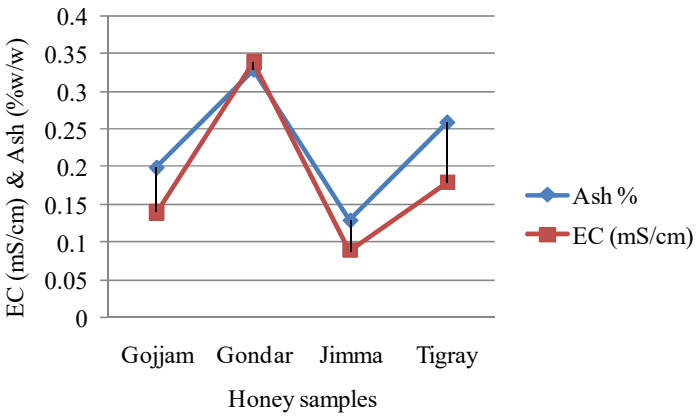

Figure 1. Relationship between ash content and EC.

As shown in Figure 1, EC increased with increasing ash content and vice versa.The ash content and EC for honey samples is in the order of Jimma < Gojjam < Tigray < Gondar, respectively. Among the studied honey samples, Gondar honey has the highest electrical conductivity and ash content, indicating highest mineral content. Electrical conductivity of honey is strictly linked with contents of their mineral salts and organic acids. Hence, lower electrical conductivity implies the lower concentration of the metals in the honey samples.

Determination of the concentration of metals in the honey samples

The concentration of the metals determined in honey samples are summarized in Table 5. Six trace heavy metals were determined in honey harvested from Gojjam, Gondar, Jimma and Tigray. The results show that the mean concentration values of the metals varied from one place to another.

Table 5. Concentrations of heavy metals in Gojjam, Gondar, Jimma and Tigray honey samples in μg/g (Mean ± SD).

Site of collection	Metals (μg/g)	
	Zn	Cu
Gojjam	10 ± 0.02	18 ± 0.08
Gondar	9.0 ± 0.01	7.1 ± 0.07
Jimma	9.7 ± 0.09	7.0 ± 0.03
Tigray	4.2 ± 0.20	6.2 ± 0.06

Four of the six trace heavy metals studied (Cd, Pb, Cr and Ni) were found below the detection limit of the instrument in the four honey samples. The very low or none of these trace toxic heavy metals in the honey samples indicates that the areas from which the honeys produced are less contaminated with the heavy metals from anthropogenic activities and therefore are safe for human consumption. Among the analyzed metals, Cu was found the most abundant trace metal and the values of Cu in honey from Gojjam, Gondar, Jimma and Tigray were within the range of 6.2 μg/g to 18 μg/g. The highest value of (18 μg/g) Cu was recorded in honey from Gojjam and the lowest from Tigray (6.2 μg/g). In this work, Zn was the second most abundant trace heavy metal next to Cu. The average values of Zn in honey from Gojjam, Gondar, Jimma and Tigray were within the range of 4.2 μg/g to 10 μg/g.

Comparison of metal levels and physicochemical properties of the four honey samples with other reported values

Although there were differences in sampling area, sample preparation, analytical techniques, and working conditions, the findings of this study can be compared with the other literature values. The physicochemical properties (pH, EC and ash content) and levels of metals (Zn and Cu) were compared with the findings of other researchers.

Table 6 shows the means and ranges of the data obtained from the analysis of the physicochemical parameters (pH, EC and ash content). The pH values of honey samples from different countries and other previous studies from Ethiopia are all acidic and comparable with the present study. The pH values are within the standard limit in agreement with the established fact that honey is characteristically acidic [34]. It is acidic due to the presence of organic acids (tartaric, citric, oxalic, acetic, etc.), nectar or bees secretions in the honey [30].

Table 6. Comparison of physicochemical properties of the present study with different countries honey.

Country	Physicochemical properties			References
	pH	EC ($mScm^{-1}$)	Ash %	
Saudi	4.46	0.53	0.23	[9]
India	3.14-4.16	NR	0.24-0.58	[36]
Nigeria	3.48-5.16	NR	0.32-0.96	[37]
Malaysia	3.21-3.50	0.15-1.64	0.19-0.27	[5, 15]
Argentina	3.34-4.70	NR	0.06-0.21	[38]
Brazil	2.93-4.08	0.51-0.72	0.14-0.32	[39]
Pakistan	3.38-4.41	0.08-0.31	NR	[2]
Ethiopia	3.82- 4.45	0.008- 0.033	0.08- 0.17	[33]
Ethiopia	3.98-4.12	0.35-0.65	0.09-0.26	[35]
Ethiopia	NR	NR	0.10-0.14	[22]
Ethiopia	3.84-4.00	0.09-0.34	0.13-0.33	Present study

NR- not reported.

The EC of honey samples from Saudi, Brazil, Pakistan and the results from this study were found slightly higher for honey from Malaysia. However, EC values of the present study are quite similar to the EC values from previous reports in Ethiopia by Nigussie *et al.* [33] and Nega *et al.* [35]. All the EC values in the current study are lower than that established by the standard Codex Alimentarius [28] (< 0.8 $mScm^{-1}$), showing that the honeys are possibly floral honey origin [33].

The ash content of honey samples from most of the countries including from previously conducted research in Ethiopia as shown in Table 6 are in a good agreement with that of the present study. All the honey samples studied have ash content below 0.6%, permitted limit for flower honeys according to the Council of the European Union [27].

The levels of the analyzed trace and toxic metals (Pb, Cd, Cr and Ni) are not presented in Table 7 as they were found below detection limit of the instrument. The concentration of Zn in the present study was found higher when compared with reported concentration values from the literatures [33, 38, 40, 41] but fairly comparable with reports from [15, 12, 41]. Similarly, Cu concentration in the current study is quite similar to the report from Ethiopia by Nigussie *et al.* [33] but different from other reported findings. The difference observed in the concentration of reported values and the present study might be due to the difference in botanical origin, climatic conditions and other factors.

Table 7. Comparison of the concentration of metals in honey of the present study with other reported values.

Country	Concentrations of metals (μg/g)		
	Zn	Cu	References
Israel	0.8-11.5	0.9-3.18	[15]
Iran	0.31-6.03	0.08-0.27	[12]
Italy	2.5-3.1.0	0.31-0.91	[38]
India	0.01-1.75	0.004-0.015	[40]
Hungary	0.18-7.2	0.002-0.78	[41]
Ethiopia	NR	0.27-0.28	[35]
Ethiopia	0.373- 1.12	0.370- 14.0	[33]
Ethiopia	NR	0.08-0.27	[22]
Present study	4.2-10	6.2-18	

NR- not reported.

Analysis of variance (ANOVA)

In this study, the variation in sample means of the levels of the metals were tested whether they have significant difference or not by using one way ANOVA at 95% confidence level ($p = 0.05$). In this study, the statistical analysis indicated that there were no significant differences among the sample mean concentrations for Cu ($p \geq 0.05$) whereas there were significant differences in the mean concentration of Zn ($p < 0.05$) in the samples (Table 8). This may be due to variations in factors other than the experimental procedures. The causes for this difference between sample means could be possibly attributed to the floral sources from which honey bees extract the nectar, climatic difference, geographical and storage factors.

Table 8. Analysis of variance (ANOVA) between and within honey samples at 95% confidence level.

Metal	Cu	Zn
F_{cal}	2.276	8.759
F_{crit}	4.066	4.066
p-value	0.1567	0.0065

CONCLUSION

The determination of the level of the metals Cd, Pb, Zn, Cr, Cu, and Ni in honey samples was carried out by flame atomic absorption spectrometry (FAAS). The result was evaluated through the recovery test and good percentage recovery of 90.0 to 95.8% was obtained for the metals identified. Statistical analysis by using one-way ANOVA at a 95% confidence level indicated that there were no significant differences among the sample mean concentrations for Cu while there were significant differences in the mean concentration of Zn in the samples. This could be possibly attributed to the differences in the floral sources of the nectar, climatic difference, geographical and storage factors. The trace heavy metals Cd, Pb, Cr and Ni were found below the detection limit in all the honey samples, indicating that the four honey samples have very low or none of the toxic metals and safe for human consumption. The pH, EC and ash contents of the honey samples were also all within the range of the standard Codex Alimentarius.

However, the authors would like to recommend additional future studies on extended quality physicochemical parameters of honey such as pollen analysis, HMF content, sugar content, antimicrobial activities, etc. and periodical follow up on harmful substances such as toxic elements and other harmful chemicals and track the development of pollutants in honeys.

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